SemenRate :objective assessment of bovine semen through CASA and flow cytometry

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Objectives

The quality of frozen semen for artificial insemination is assessed by semen production centres (SPCs) prior to releasing semen commercially. There is generally no further assessment of semen quality before use.

Several methods can be used to evaluate the quality of fresh or frozen/thawed semen: subjective evaluation using standard optical microscopy is the most common. Parameters routinely examined are concentration, progressive motility and morphology. This subjective approach used during quality control at SPCs or investigations of poor reproductive performance in veterinary practice has been shown to be relatively inaccurate, imprecise and operator dependent (Vincent *et al*, 2012).

Semen storage and handling practices can therefore potentially impact on semen quality prior to and at insemination and therefore there was a need to assess how this was being performed on farms.

The aim of this project was to investigate the variation in quality of AI semen straws stored and in use on 50 initial farms across Yorkshire, UK. Samples were assessed using a combination of computer assisted semen analysis (CASA) and flow cytometry (FC). Another aim was to objectively assess the quality of fresh semen collected from bulls as part of breeding soundness evaluation, that had been assessed subjectively at bull side on farm. The overall aim was to produce a quick, repeatable and objective semen analysis service for farmers and veterinarians using the CASA and FC.

Currently there is no other commercially available independent service offering computerised semen analysis to cattle farms in the UK.

Methods

A funded research study has provided the initial platform to launch a commercial service:

Analyse frozen/thawed and freshly collected, extended bull semen from 50 Yorkshire cattle farms using CASA and flow cytometer (Table 1-3). Data was cross-checked by veterinary surgeons with semen evaluation experience

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Compare semen analysis parameters to subsequent fertility performance on farm to build a multi-parametric database of results (with eight European partners) to enable analysis of the parameters of most significance in terms of subsequent fertility outcomes on farm

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Extend a commercial service more widely across the UK and the EU

Freshly collected bull semen
Chilled with semen extender and posted to the centre via next day delivery for evaluation (alongside a bull breeding soundness exam)

Frozen/thawed semen,
Collected via a national network of recognized breeding centres associated with XLvets veterinary practices.
- Pre-release
- Post-purchase
- After significant periods of transport
- After storage in unregulated conditions on farm
- Pre-ET/OPU-IVF programme
- Investigation of fertility issues

Semen from other species
- porcine
- equine semen

Collaborators:
University of Glasgow; Innovate UK

Results – Pilot Survey Data

	% Viable	% Polarised Mitochondria	% Intact Acrosome	% Motile	% Prog Motile
Min	0	0.26	0.06	0	0
Max	67.64	72.50	68.82	66.9	59.00
Mean	43.44	38.56	35.29	37.44	26.11

Table 1. Frozen AI Semen Survey (n= 79)

Frozen AI semen results (Table 1) demonstrate a large range in all parameters measured, varying from semen with values close to zero for all assessments to extremely good quality AI semen with values of > 60 % from multiple samples analysed from some farms. The pre-release standards for SPCs for motility of greater than 30% have been achieved in 58 of the samples, but in 21 of the straws tested (~25%) the motility was below this standard. These straws ,therefore, are unlikely to perform as expected in the field (Phillips *et al* 2004). These data challenge the assumption that semen stored on farm is always of optimum quality.

	% Viable	% Polarised Mitochondria	% Intact Acrosome	% Motile	% Prog Motile
Min	1.03	0.78	4.98	1.40	0
Max	92.2	91.56	87.44	90.80	76.60
Mean	52.03	51.66	40.16	52.14	38.10

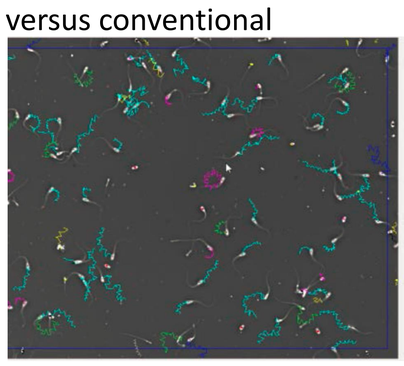
Table 2. Fresh Semen Survey(n= 67)

The fresh extended semen collected as part of routine bull breeding soundness evaluations also demonstrated great variation in all parameters measured (Table 2). Some samples did not survive transport from farm to lab in chilled polystyrene insulated boxes whilst other samples maintained excellent quality in the 12-18 hours (no drop in motility) between collection on farm and assessment in the lab. An independent external lab may be appropriate to assess semen quality as long as chilling and transport protocols are adhered to. The benefit of using the CASA and FC assessment being that they are **OBJECTIVE, REPEATABLE and AVAILABLE FOR REVIEW** in the future if any disputes regarding a bulls ability to perform arise.

	% Viable	% Polarised Mitochondria	% Intact Acrosome	% Motile	% Prog Motile
Min	17.08	13.40	15.34	9.40	3.90
Max	66.31	26.74	52.62	38	22.80
Mean	43.57	19.96	37	24.88	13.15

Table 3. Sexed Frozen AI Semen Survey (n= 9)

The sexed semen data (Table 3) do not provide evidence that mean sperm viability or acrosome integrity measurements to differ from conventional AI semen. Mean mitochondrial activity and progressive motility values drop by 50% compared with conventional semen. Alongside decreased sperm dosage in sexed semen, this may contribute to the challenge of achieving reproductive targets on farm using sexed versus conventional semen.



Conclusions

- The use of flow cytometry in genuinely multi-parametric analysis is not a standard procedure in SPCs in the UK. CASA technologies are principally used by breeding/semen companies to assess semen to ensure minimum standards prior to commercial release.
- SemenRate utilises CASA and FC to offer an independent, objective and repeatable semen analysis service to vets and farmers. The results of semen analysis are clearly only part of an overall fertility solution on farm. As well as the technical laboratory based skills required to run the analysis and scientific knowledge required to interpret results, the veterinary herd health input required to identify and manage risks associated with poor semen quality on farm are needed in partnership with the referring veterinary surgeon. Solutions to the issues causing poor quality semen may be identified and corrected, resulting in use of better quality semen in the future. Alternatively managing the risk of suboptimal semen usage can help minimise the impact on fertility on that farm. For example, a farmer may be advised to use extra straws of semen per insemination of batches found to have 'compensable' defects. Likewise appropriate advice on semen identified as having 'non-compensable' defects would be given (Hudson *et al,* 2012)
- The correlation between semen quality parameters and field fertility is not assessed currently in the UK, so now an EU collaboration will aim to establish unique data relevant to UK dairy and beef herd as well as for the EU. These original data should highlight to fellow stakeholders in the industry how imperative optimal semen quality is and the benefits to herd fertility and financial performance.
- The data collected in this pilot study are being expanded through ongoing work and statistical analysis will be reported. Sellem et al (2015) described the statistically significance association between data collected with this approach and field fertility outcomes.

References

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